SIMMESN

SOCIETÀ ITALIANA PER LO STUDIO DELLE MALATTIE METABOLICHE EREDITARIE E LO SCREENING NEONATALE

(ITALIAN SOCIETY FOR THE STUDY OF INHERITED METABOLIC DISEASES AND NEWBORN SCREENING)

MSITA

Italian Working Group on Mass Spectrometry

PROFICENCY TESTING FOR ACYLCARNITINES AMINOACIDS DBS

Year 2020

1. General information

Data presented here derives from data submitted by the labs to the CRB website.

1.1 Participants, materials and program methods

Thirty-four labs are enrolled in the program as follows: Italy (n=16), Spain (n=15), Portugal (n=1), Switzerland (n=1), Netherlands (n=1).

Fig. 1 indicates the % of lab distribution by country.

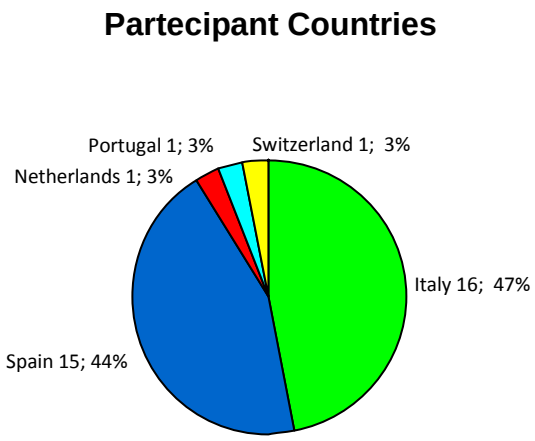


Fig. 1-The % of labs distribution by country

The participation is anonymous (each lab is identified by a code) and free of charge. All the costs are sustained by the SIMMESN and CRB.

Biological material consists of human blood, collected as dried blood spots (DBSs) on 903 grade paper (or equivalent) and stored at –20° Celsius.

All participants are requested to submit biological material from real patients affected with a metabolic defect suitable for expanded newborn screening. Submitters are asked to declare the abnormalities found on the sample and have the informed consent to collect and circulate the material.

It must be stressed that the biological material doesn't have to necessarily come from a newborn and that the patient could be in treatment.

The submission of material is crucial for the program so as to have enough biological material to proceed in the following years.

A set of three DBSs was sent by express courier to each lab in April and the participants were requested to send the results before 17th July 2020 via the CBR website:

http://centroricercabiomedica.net/.

A website page is available for information and reports: http://centroricercabiomedica.net/.

Data is password protected.

The final report will be available in SIMMESN website: https://www.simmesn.it/.

In 2020 results were returned by 32 labs (94%): 1 Italian lab and 1 Spanish lab didn't return any results.

1.2 Instruments and analytical methods

As declared by participants, a wide variety of LC-MS/MS instruments were used:

Waters TQD	11
Waters Xevo	3
Waters Quattro micro	1
Waters Quattro Ultima	1
Sciex API 4500	2
Sciex API 4000	3
Sciex API 3200	3
Sciex QTRAP 5500	1
Perkin Elmer Q Sight	4
Shimadzu LCMS 8060	1
Not declared	2

Nine labs out of 32 measured aminoacids and acylcarnitines as butyl-esters (28%), the others used an underivatized procedure.

Twenty-five labs used a commercial kit, 5 labs didn't use a kit.

As declared by participants, a wide variety of kits were used:

Perkin Elmer Neo Base	8
Perkin Elmer Neo Base2	12
Perkin Elmer Neo Gram	1
Chromosystems Mass Chrom kit	3
NeoMass AAAC	1
MS/MS non-kit	5
Not declared	2

2. Results DBS 2020-A

This sample was obtained from a five years old male diagnosed at birth by NBS.

Significant elevation of C5DC-carnitine or C5DC+C6OH-carnitine had been found. Most likely diagnosis is: **Glutaric acidemia type I (Omim 231670)**.

Table 1 shows the measurements obtained by all the labs for C5DC or C5DC+C6OH-carnitines.

Lab code	C5DC	C5DC cut off	C5DC+C6OH	C5DC+C6OH cut off
CRB_001			2,99	0,25
CRB_002	0,85	0,17		
CRB_004			3,06	0,23
CRB_005			3,57	0,33
CRB_006			3,68	0,30
CRB_007			3,51	0,20
CRB_008			0,96	0,07
CRB_009			3,94	0,26
CRB_010	4,18	0,14		
CRB_012			3,19	0,30
CRB_013	1,76	0,12		
CRB_014			4,25	0,22
CRB_015			2,80	0,10
CRB_016			3,34	0,15
CRB_017			3,04	0,18
CRB_018	2,89	0,15		
CRB_019			4,03	0,27
CRB_020	6,77	0,25		
CRB_021	2,05	0,28		
CRB_022			2,79	0,11
CRB_023			3,39	0,35
CRB_024	2,99	0,09		
CRB_025			4,12	0,85
CRB_032			3,29	0,15
CRB_035			4,17	0,26
CRB_036	5,34	0,18		
CRB_037			2,61	0,16
CRB_038			3,93	0,26
CRB_039			2,44	0,27
CRB_040	3,82	0,37		
CRB_041			3,12	0,18
CRB_042			3,71	0,27
Average	**3,41**	**0,19**	**3,30**	**0,25**
Median	**2,99**	**0,17**	**3,34**	**0,25**
SD	**1,85**	**0,09**	**0,73**	**0,15**
CV%	**54,29%**	**45,79%**	**21,97%**	**60,56%**
1Q-3Q	**2,05-4,18**	**0,14-0,25**	**3,02-3,82**	**0,17-0,27**
range	**0,85-6,77**	**0,09-0,37**	**0,96-4,25**	**0,07-0,85**
num lab	**9**	**9**	**23**	**23**

Table 1 - Sample 2020-A.Measures are expressed as µmol/L. Labs in green used derivatized method. Lowest and highest values are enhanced. Empty cells indicate data not reported.

One lab declared to perform second tier test for C4DC+CH5OH.

Suggested diagnosis as submitted by the participants are shown in Table 2.

Lab code	Suggested diagnosis	Alternative diagnosis
CRB_001	Glutaric Acidemia Type I (GAI)	
CRB_002	Glutaric Acidemia Type I (GAI)	
CRB_004	Glutaric Acidemia Type I (GAI)	
CRB_005	Glutaric Acidemia Type I (GAI)	
CRB_006	Glutaric Acidemia Type I (GAI)	
CRB_007	Glutaric Acidemia Type I (GAI)	
CRB_008	Glutaric Acidemia Type I (GAI)	
CRB_009	Glutaric Acidemia Type I (GAI)	Glutaric Acidemia Type II (GAII)
CRB_010	Glutaric Acidemia Type I (GAI)	
CRB_012	Glutaric Acidemia Type I (GAI)	Glutaric Acidemia Type II (GAII)
CRB_013	Glutaric Acidemia Type I (GAI)	
CRB_014	Glutaric Acidemia Type I (GAI)	Glutaric Acidemia Type II (GAII)
CRB_015	Glutaric Acidemia Type I (GAI)	
CRB_016	Glutaric Acidemia Type I (GAI)	
CRB_017	Glutaric Acidemia Type I (GAI)	
CRB_018	Glutaric Acidemia Type I (GAI)	
CRB_019	Glutaric Acidemia Type I (GAI)	
CRB_020	Glutaric Acidemia Type I (GAI)	
CRB_021	Glutaric Acidemia Type I (GAI)	
CRB_022	Glutaric Acidemia Type I (GAI)	
CRB_023	Glutaric Acidemia Type I (GAI)	
CRB_024	Glutaric Acidemia Type I (GAI)	
CRB_025	Glutaric Acidemia Type I (GAI)	
CRB_032	Glutaric Acidemia Type I (GAI)	
CRB_035	Glutaric Acidemia Type I (GAI)	
CRB_036	Glutaric Acidemia Type I (GAI)	
CRB_037	Glutaric Acidemia Type I (GAI)	
CRB_038	Glutaric Acidemia Type I (GAI)	
CRB_039	Glutaric Acidemia Type I (GAI)	
CRB_040	Glutaric Acidemia Type I (GAI)	
CRB_041	Glutaric Acidemia Type I (GAI)	
CRB_042	Glutaric Acidemia Type I (GAI)	

Table 2. Sample 2020-A. Suggested diagnosis.

Thirty-two labs (100%) considered **Glutaric acidemia type I** as the most likely diagnosis. Just 3 (9,4%) labs suggested in addition, the possibility of **Glutaric acidemia type II.**

Suggested follow up tests to confirm the diagnosis or guide further investigation were: urinary organic acids analysis (n=22), plasma acylcarnitines analysis (n=7), molecular analysis (n=15, 10 labs specified GCDH gene).

One laboratory mentioned the dosage of urinary glutarylcarnitine as confirmatory analysis (see reference 1).

Comment

Glutaric aciduria type I (GA-I) is a rare inherited metabolic disease caused by deficiency of glutaryl-CoA-dehydrogenase (GCDH): this enzyme catalyzes the conversion of glutaryl-CoA to crotonyl-CoA and it is located in the catabolic pathways of L-lysine, L-hydroxylysine, and L-tryptophan (see references 2 and 3).

NBS for GA-I primarily relies on measuring glutarylcarnitine (**C5DC**) in dried blood spots, which has been shown to have 96% sensitivity. Positive C5DC values require follow-up biochemical testing with either urine organic analysis or quantitative glutaric and 3-hydroxyglutaric acid, with preference for quantitative studies if available (see reference 4).

Preliminary laboratory findings include significantly elevated concentrations of the following metabolites using gas chromatography/mass spectrometry or electrospray-ionization tandem mass spectrometry: Glutaric acid, 3-hydroxyglutaric acid, Glutarylcarnitine (C5DC) and Glutaconic acid (see reference 4).

All the labs found C5DC-carnitine or C5DC+C6OH-carnitine values above the reported cut-off.

With particular reference to quantitative dosage, C5DC+C6OH-carnitine interlabs CV% value was lower than C5DC-carnitine interlabs CV% value (range 0,81-6,77 and 0,56-4,25 respectively). High variability of C5DC-carnitine is ascribable to the use of different internal standard.

Anyway, the results for this sample are certainly satisfactory.

Reference:

1) Tortorelli S, Hahn SH, Cowan TM, Brewster TG, Rinaldo P, Matern D. The urinary excretion of glutarylcarnitine is an informative tool in the biochemical diagnosis of glutaric acidemia type I. Mol Genet Metab. 2005;84(2):137-143. doi:10.1016/j.ymgme.2004.09.016

2) Shadmehri AA, Fattahi N, Pourreza MR, et al. Molecular genetic study of glutaric aciduria, type I: Identification of a novel mutation. J Cell Biochem. 2019;120(3):3367-3372. doi:10.1002/jcb.27607

3) Boy N, Mühlhausen C, Maier EM, et al. Proposed recommendations for diagnosing and managing individuals with glutaric aciduria type I: second revision. J Inherit Metab Dis. 2017;40(1):75-101. doi:10.1007/s10545-016-9999-9

4) Larson A, Goodman S. Glutaric Acidemia Type 1. 2019 Sep 19. In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2020

3. Results DBS 2020-B

This sample comes from a six years old female diagnosed at the age of 6 months. Patient was admitted for acidosis, encephalopathy, acranocyanosis and psychomotor retardation. Liver transplant at 9 months.

Significant elevation of C4-carnitine and slightly increase of C5-carnitine have been found at the first step. Most likely diagnosis is **Ethylmalonic encephalopathy (Omim 602473)**.

Table 3 shows the measurements obtained by all the labs for C4 and C5-carnitines.

Lab code	C4	C4 cut off	C5	C5 cut off
CRB_001	1,26	0,50	0,49	0,23
CRB_002	1,32	0,70	0,32	0,40
CRB_004	1,62	0,76		0,30
CRB_005	1,38	0,68	0,62	0,38
CRB_006	1,47	0,62	0,63	0,32
CRB_007	1,04	0,90	0,57	0,35
CRB_008	1,10	0,62	0,54	0,26
CRB_009	1,22	0,69	0,54	0,34
CRB_010	1,54	0,88	0,52	0,35
CRB_012	1,33	0,80	0,57	0,35
CRB_013	1,63	0,90	0,56	0,26
CRB_014	1,56	0,48	0,59	0,60
CRB_015	1,17	0,62	0,49	0,26
CRB_016	1,37	0,63	0,62	0,24
CRB_017	1,30	0,63	0,59	0,25
CRB_018	1,20	0,72	0,60	0,43
CRB_019	1,08	0,87	0,42	1,10
CRB_020	1,44	0,64	0,68	0,50
CRB_021	1,63	0,73	0,68	0,64
CRB_022	1,43	0,67	0,64	0,54
CRB_023			0,47	0,80
CRB_024	1,60	0,51	0,56	0,25
CRB_025	1,40	1,10	0,55	0,50
CRB_032	1,57	0,43	0,62	0,40
CRB_035				
CRB_036	1,26	0,46	0,56	0,24
CRB_037	1,08	1,08	0,46	0,39
CRB_038	1,42		0,58	
CRB_039	1,25	0,95	0,49	0,80
CRB_040	1,43		0,48	0,79
CRB_041	1,38	0,81	0,49	0,49
CRB_042	1,31	0,59	0,57	0,60
Average	**1,36**	**0,71**	**0,55**	**0,45**
Median	**1,38**	**0,69**	**0,56**	**0,39**
SD	**0,17**	**0,18**	**0,08**	**0,21**
CV%	**12,7**	**24,6**	**14,2**	**47,5**
1Q-3Q	**1,25-1,46**	**0,62-0,83**	**0,49-0,60**	**0,27-0,53**
range	**1,04-1,63**	**0,43-1,10**	**0,32-0,68**	**0,23-1,10**
num lab	**30**	**28**	**30**	**30**

Table 3 - Sample 2020-B- Measures are expressed as µmol/L. Values in yellow cells were below the reported cut-off. Lowest and highest values are enhanced. Empty cells indicate data not reported

Only 6 labs performed second tier test for ethylmalonic acid. In table 4 obtained results are reported.

Lab code	Ethylmalonic acid	Ethylmalonic acid cut off
CRB_001	2,30	10,00
CRB_006	1,30	2,60
CRB_008	2,60	
CRB_010	3,30	1,50
CRB_013	1,60	3,70
CRB_017	12,50	3,00

*Table 4. Second tier test results for ethylmalonic acid. Measures are expressed as µmolL.. Values **in yellow** cells were below the reported cut-off. **Lowest and highest** values are enhanced. Empty cells indicate data not reported*

Three out of 6 labs found a normal ethylmalonic acid concentration on DBS and one lab suggested it could be due to liver transplantation.

Suggested diagnosis are shown in Table 5 as submitted by the participants.

Lab code	Suggested diagnosis	Alternative diagnosis
CRB_001	Ethylmalonic encephalopathy (EE)	Glutaric Acidemia Type II (GAII)
CRB_002	Ethylmalonic encephalopathy (EE)	
CRB_004	Ethylmalonic encephalopathy (EE)	
CRB_005	Ethylmalonic encephalopathy (EE)	Multiple Carboxylase Deficiency (MCD)
CRB_006	Ethylmalonic encephalopathy (EE)	
CRB_007	Ethylmalonic encephalopathy (EE)	Glutaric Acidemia Type II(GAII)
CRB_008	Ethylmalonic encephalopathy (EE)	
CRB_009	Medium/Short Chain Acyl-CoA Dehydrogenase (M/SCHAD)	Glutaric Acidemia Type II (GAII)
CRB_010	Ethylmalonic encephalopathy (EE)	Glutaric Acidemia Type II (GAII)
CRB_012	Ethylmalonic encephalopathy (EE)	Isovaleric Acidemia (IVA)
CRB_013	Ethylmalonic encephalopathy (EE)	Glutaric Acidemia Type II (GAII)
CRB_014	Ethylmalonic encephalopathy (EE)	Short chain acyl-CoA-dehydrogenase deficiency (SCAD)
CRB_015	Ethylmalonic encephalopathy (EE)	
CRB_016	Ethylmalonic encephalopathy (EE)	Short chain acyl-CoA-dehydrogenase deficiency (SCAD)
CRB_017	Ethylmalonic encephalopathy (EE)	
CRB_018	Glutaric Acidemia Type II (GAII)	
CRB_019	Ethylmalonic encephalopathy (EE)	Isobutyril-CoA dehydrogenase deficiency (IBD)
CRB_021	Short chain acyl-CoA-dehydrogenase deficiency (SCAD)	Glutaric Acidemia Type II (GAII)
CRB_022	Ethylmalonic encephalopathy (EE)	
CRB_023	Normal	
CRB_024	Glutaric Acidemia Type II (GAII)	
CRB_025	Normal	
CRB_032	Ethylmalonic encephalopathy (EE)	Short chain acyl-CoA-dehydrogenase deficiency (SCAD)
CRB_035		
CRB_036	Ethylmalonic encephalopathy (EE)	2methylbutyril-CoA dehydrogenase deficiency (2MBG)
CRB_037		
CRB_038		
CRB_039	Short chain acyl-CoA-dehydrogenase deficiency (SCAD)	
CRB_040		
CRB_041		
CRB_042	Short chain acyl-CoA-dehydrogenase deficiency (SCAD)	Isobutyril-CoA dehydrogenase deficiency (IBD)

Table 5. Sample 2020-B. Suggested diagnosis.

Twenty-two out of 31 (71%) labs reported simultaneous increase of C4-carnitine and C5-carnitine levels. 6 labs reported only C4-carnitine elevation.

Eighteen labs suggested Ethylmalonic Encephalopathy (EE) diagnosis and three labs suggested Short chain acyl-dehydrogenase deficiency (SCAD), 1 lab suggested Glutaric acidemia type II (GAII) and 1 lab suggested Medium/Short chain acyl-CoA dehydrogenase (M/SCHAD). Two labs found a normal profile for this sample.

Suggested follow up tests to confirm the diagnosis or guide further investigation were: molecular analysis (n=16, 11 labs specified ETHE1 gene), urinary organic acids analysis (n=24), plasma acylcanitines analysis (n=7), urinary acylglycines analysis (n=2), MRI (n=1).

Comment

Ethylmalonic encephalopathy (EE) is a rare, multisystem infantile autosomal recessive disorder caused by mutations in ETHE1.

EE presents with developmental delay, diarrhea and petechiae (see references 1, 2 and 3).

In EE, blood and plasma acylcarnitine analysis will typically show increased C4- and C5-acylcarnitines and urine organic acids/acylglycine analysis will show increased ethylmalonic acid isovalerylglycine, isobutirylglicine and 2-methylbutyrylglicine.

The EE acylcarnitine profile can be differentiated from SCAD, Isobutyryl-CoA dehydrogenase (IBD) in which C4-carnitine alone is increased and 3-Hydroxyacyl-CoA dehydrogenase deficiency (HAD) in which C4-carnitine and C4OH-carnitine are both increased (see references 2).

Furthermore, an increase in C4-carnitine when associated with an increase in C5, C6, C8, C10, C14, C14, C14:1, C5DC carnitines may indicate a Multiple acyl-CoA dehydrogenase deficiency (GAII) (see references 4).

Anyway, the results for this sample are certainly satisfactory.

Reference:

1) Burlina A, Zacchello F, Dionisi-Vici C, et al. New clinical phenotype of branched-chain acyl-CoA oxidation defect. Lancet. 1991;338(8781):1522-1523. doi:10.1016/0140-6736(91)92338-3

2) Burlina AB, Dionisi-Vici C, Bennett MJ, et al. A new syndrome with ethylmalonic aciduria and normal fatty acid oxidation in fibroblasts. J Pediatr. 1994;124(1):79-86. doi:10.1016/s0022-3476(94)70257-8

3) Dionisi-Vici C, Diodato D, Torre G, et al. Liver transplant in ethylmalonic encephalopathy: a new treatment for an otherwise fatal disease.Brain. 2016;139(Pt 4):1045-1051. doi:10.1093/brain/aww013

4) N K Poplawski 1, E Ranieri, J R Harrison, J M Fletcher Multiple acyl-coenzyme A dehydrogenase deficiency: diagnosis by acyl-carnitine analysis of a 12-year-old newborn screening card. 1999 Jun;134(6):764-6.

4. Results DBS 2020-C

This DBS comes from a twenty-three years old male diagnosed at 2 weeks of life. Patient with neonatal hyperammonemia, actually under treatment and diet.

Significant elevation of Argininosuccinic Acid (ASA) and moderately increase of Citrulline have been found. Most likely diagnosis is **Argininosuccinic Aciduria (Omim 207900)**.

Table 6 shows the measurements submitted for Argininosuccinic acid and Citrulline and the related cut-off values.

Lab code	ASA	ASA cut off	Citrulline	Citrulline cut off
CRB_001	23,89	1,00	111,0	40,0
CRB_002			114,4	30,0
CRB_004	25,98	0,60	110,4	35,0
CRB_005	10,64	0,15	122,7	40,0
CRB_006	36,30	0,90	145,0	28,1
CRB_007			111,4	45,0
CRB_008	14,35	0,50	83,2	46,0
CRB_009	25,70	0,89	137,3	32,0
CRB_010	246,40	3,90	150,0	50,0
CRB_012	30,60	1,00	129,0	35,0
CRB_013	10,88	0,24	95,3	20,0
CRB_014	17,58	0,50	116,4	41,0
CRB_015	30,00	1,00	128,0	46,0
CRB_016	19,70	1,20	115,0	23,8
CRB_017	28,75	1,04	127,5	30,0
CRB_018			109,9	25,2
CRB_019			123,7	49,0
CRB_020	114,0	2,50	136,0	32,6
CRB_021	30,1	0,89	132,0	34,2
CRB_022	21,0	0,90	116,0	59,2
CRB_023				
CRB_024	4,5	0,27	100,9	26,5
CRB_025			145,0	30,0
CRB_032			143,4	64,3
CRB_035				
CRB_036			144,3	29,7
CRB_037	21,4	0,78	93,4	39,2
CRB_038			121,0	
CRB_039	38,4	3,59	126,0	55,0
CRB_040			145,2	
CRB_041				
CRB_042			119,3	
Average	**39,49**	**1,15**	**122,5**	**38,0**
Median	**25,70**	**0,90**	**122,7**	**35,0**
SD	**54,98**	**1,04**	**17,1**	**11,3**
CV%	**139,22**	**90,66**	**13,9**	**29,7**
1Q-3Q	**18,6-30,3**	**0,55-1,02**	**111,4-136,0**	**30,0-45,7**
range	**4,5-246,4**	**0,15-3,90**	**83,2-150,0**	**20,0-64,3**
num lab	19	19	29	26

Table 6 - Sample 2020-C. Measures are expressed as µmol/L. Lowest and highest values are enhanced. Empty cells indicate data not reported

Suggested diagnosis are shown in Table 7 as submitted by the participants.

Lab code	Suggested diagnosis	Alternative diagnosis
CRB_001	Argininosuccinic aciduria (ASA)	
CRB_002	Argininosuccinic aciduria (ASA)	
CRB_004	Argininosuccinic aciduria (ASA)	
CRB_005	Argininosuccinic aciduria (ASA)	
CRB_006	Argininosuccinic aciduria (ASA)	
CRB_007	Argininosuccinic aciduria (ASA)	Citrullinemia type II (CIT II)
CRB_008	Argininosuccinic aciduria (ASA)	
CRB_009	Argininosuccinic aciduria (ASA)	
CRB_010	Argininosuccinic aciduria (ASA)	
CRB_012	Argininosuccinic aciduria (ASA)	
CRB_013	Argininosuccinic aciduria (ASA)	
CRB_014	Argininosuccinic aciduria (ASA)	
CRB_015	Argininosuccinic aciduria (ASA)	
CRB_016	Argininosuccinic aciduria (ASA)	
CRB_017	Argininosuccinic aciduria (ASA)	
CRB_018	Citrullinemia type I (CIT I)	Argininosuccinic aciduria (ASA)
CRB_019	Citrullinemia type I (CIT I)	Argininosuccinic aciduria (ASA)
CRB_020	Argininosuccinic aciduria (ASA)	
CRB_021	Argininosuccinic aciduria (ASA)	
CRB_022	Argininosuccinic aciduria (ASA)	
CRB_023	Normal	
CRB_024	Argininosuccinic aciduria (ASA)	
CRB_025	Citrullinemia type I (CIT I)	
CRB_032	Citrullinemia type I (CIT I)	Argininosuccinic aciduria (ASA)
CRB_035		
CRB_036	Citrullinemia type II (CIT II)	Citrullinemia type I (CIT I)
CRB_037	Argininosuccinic aciduria (ASA)	
CRB_038	Citrullinemia type I (CIT I)	
CRB_039	Argininosuccinic aciduria (ASA)	
CRB_040		
CRB_041		
CRB_042		

Table 7 – Sample 2020-C. Suggested diagnosis.

Twenty-one labs (68%) suggested Argininosuccinic aciduria as possible diagnosis. Five labs suggested Citrullinemia type I and one lab Citrullinemia type II. One lab reported this sample as normal. Three laboratories did not suggest any diagnosis.

Further suggested alternative diagnoses: Citrullinemia type II (1 lab), Citrullinemia type I (1 lab), Argininosuccinic aciduria (3 labs).

Suggested follow up tests to confirm the diagnosis or guide further investigation were:

plasma aminoacids analysis (n=20), urinary aminoacids analysis (n=10), mutation analysis (n=11, all of labs specified ASL gene), urinary organic acids analysis (n=5), enzyme assay in cultured fibroblasts (n=4), urinary orotic acid analysis (n=8), ammonia (n=2).

Comment

Argininosuccinic aciduria (ASA) is caused by a deficient function of argininosuccinate lyase (ASL), which catalyses the transformation of argininosuccinate into arginine, an essential reaction for the waste of excessive nitrogen through the urea cycle and endogenous arginine synthesis (see reference 1).

NBS for ASL deficiency is primarily based on quantification of the analytes argininosuccinic acid and citrulline on dried blood spots (see reference 2).

Elevated plasma ammonia concentration (>100 µmol/L), elevated plasma citrulline concentration (usually 100-300 µmol/L), and elevated argininosuccinic acid in plasma or urine establish the diagnosis of ASA (see reference 2).

Anyway, the results for this sample are certainly satisfactory.

Reference:

1) Baruteau J, Diez-Fernandez C, Lerner S, Ranucci G, Gissen P, Dionisi-Vici C, Nagamani S, Erez A, Häberle J. Argininosuccinic aciduria: Recent pathophysiological insights and therapeutic prospects. J Inherit Metab Dis. 2019 Nov;42(6):1147-1161. doi: 10.1002/jimd.12047. Epub 2019 Feb 5. PMID: 30723942.

2) Nagamani SCS, Erez A, Lee B. Argininosuccinate Lyase Deficiency. 2011 Feb 3 [updated 2019 Mar 28]. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2020. PMID: 21290785.

5. Scoring

Criteria for scoring are:

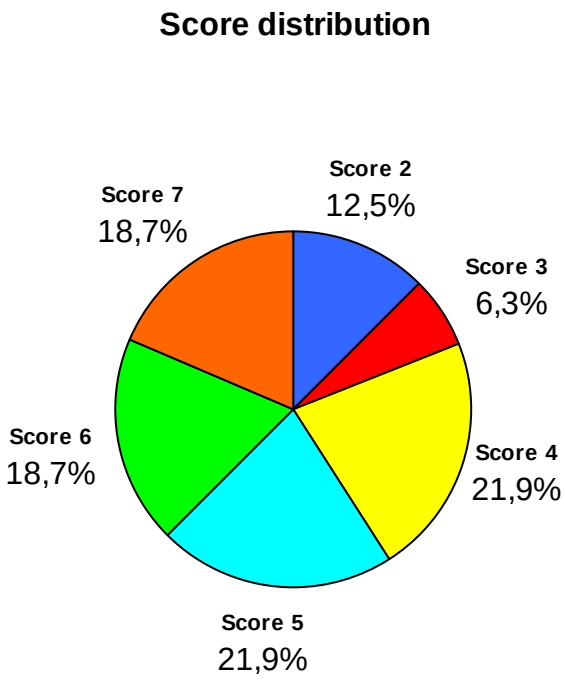
R = Right Classification- complete: 2 points
H = Right Classification- Incomplete: 1 point
W = Wrong classification: 0 points
N = Not submitted: 0 points
2nd-t Second-tier results (if available): 1 point

The maximum achievable score was 7 points as maximum. Table 8 reports the scores for all DBSs for each Lab.

Lab code	DBS 2020-A	DBS 2020-B	DBS 2020-C	Total score
CRB_001	2	3	2	7
CRB_002	2	1	1	4
CRB_004	2	1	2	5
CRB_005	2	2	2	6
CRB_006	2	3	2	7
CRB_007	2	2	1	5
CRB_008	2	3	2	7
CRB_009	2	1	2	5
CRB_010	2	3	2	7
CRB_012	2	2	2	6
CRB_013	2	3	2	7
CRB_014	2	1	1	4
CRB_015	2	2	2	6
CRB_016	2	2	2	6
CRB_017	2	3	2	7
CRB_018	2	1	1	4
CRB_019	2	1	1	4
CRB_020	2	2	2	6
CRB_021	2	1	2	5
CRB_022	2	2	2	6
CRB_023	2	0	0	2
CRB_024	2	1	2	5
CRB_025	2	1	1	4
CRB_032	2	2	1	5
CRB_035	2	0	0	2
CRB_036	2	2	0	4
CRB_037	2	0	2	4
CRB_038	2	0	0	2
CRB_039	2	1	2	5
CRB_040	2	0	0	2
CRB_041	2	1	0	3
CRB_042	2	1	0	3

Table 8 – Labs' scoring.

Fig. Score distribution.



Our project can only continue if we have enough biological material to distribute.

All participants are strongly requested to submit positive samples according to the procedure indicated on the website.